

SCREENING OF PHILIPPINE MEDICINAL PLANTS FOR ANTICANCER AGENTS USING CCNSC PROTOCOLS^{1,2}Victoria A. Masilungan,³ S. Vadlamudi,⁴ and Abraham Goldin⁵

SUMMARY

Extracts of 7 species of plants used locally in the Philippines to treat cancer were screened for the presence of antitumor activity in leukemia L1210, leukemia P388, Sarcoma 180, Adenocarcinoma 755, and Walker carcinosarcoma 256 (intramuscular). Although treatment with extracts of all of the plants resulted in some inhibition of growth in one or more of the tumor systems, none of the plant extracts met the criteria for effectiveness established for these screens in the program of the Cancer Chemotherapy National Service Center.

The Cancer Chemotherapy National Service Center (CCNSC) has published 13 reports (1) dealing with plant extracts which failed to demonstrate sufficient activity in one or more primary screening systems to warrant additional investigation. In the most recent report of Abbott et al (1) almost all of a variety of plant extracts were tested against Sarcoma 180 (S180), Adenocarcinoma 755 (Ca755), leukemia L1210 (L1210), and KB cells in culture. Some tests were also done with Dunning ascites leukemia, Lewis lung carcinoma, Walker carcinosarcoma 256 (intramuscular), human sarcoma HSI, Friend virus leukemia, P1798 lymphosarcoma, and Murphy-Sturm lymphosarcoma. In these screening studies the extracts were derived from plants collected from many parts of the world; however, no collections were made from the Philippines. Nonetheless, some of the extracts used came from the same species of plants reported by Masilungan et al (2) to possess some anticancer activity against Ehrlich ascites tumor cells. Since the constitution of a plant may be influenced by alterations in geographic locale including attendant variations in soil, water, temperature, sunlight, etc, it was considered of interest to conduct additional screening tests with extracts from Philippine medicinal plants listed by Quisumbing (3), and used locally against a variety of malignant diseases.

In the present study the extracts of Philippine medicinal plants were tested for their inhibitory effect on L1210, S180, Ca755, Walker carcinosarcoma 256 (Walker 256) inoculated intramuscularly (im), and leukemia P388 (P388).

MATERIALS AND METHODS

The 7 species of plants listed in table 1 were collected in San José, Batangas, Philippines. The selection of solvent for preparing the extracts was based on the studies of Masilungan et al (2).

Alcoholic extracts of fresh leaves of *Cinnamomum zeylanicum*, *Vitex negundo*, or *Aristolochia tagala* were prepared by macerating 1 kg of ground leaves of each plant in 4 liters of alcohol. After standing for 24 hours at room temperature the extract was filtered and the filtrate evaporated at low temperature to a syrupy consistency. Extracts from the leaves of *Erythrina variegata* Linn. var. *orientalis* and seeds of *Cucumis melo* were also prepared using the above procedure except that 1% HCl was used instead of alcohol. For the leaves of *Viola odorata*, 1% sodium bicarbonate was used instead of alcohol in preparing the extract. An ether extract from the seeds of *Raphanus sativus* was prepared using a Soxhlet extraction apparatus. The ether was then evaporated leaving an oily extract.

In the preparation of an injectable form, vehicles used by the CCNSC were tested as possible diluents for each extract. Selection of the vehicle for each extract (table 1) was based on its capacity for dissolving or suspending the extract. The concentrations of prepared extracts for injection were computed on the basis of moisture-free samples. Alkaline or acid solutions or suspensions of the extracts were adjusted to pH 6-9.

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The 5 transplant tumor systems used for screening the extracts (L1210, S180, Ca755, Walker 256, and P388) have been used extensively at the CCNSC. The detailed CCNSC protocols were used for each tumor system (4).

Tumor transplantation was done under aseptic conditions. The tumor fragments and tumor cell suspensions were tested for sterility in each experiment using tubes of thioglycollate broth.

The details of the treatment procedures for each test are included with each line of summary data in table 1. The materials were injected either intraperitoneally (ip) or subcutaneously (sc). The dosage schedule involved one injection daily but the number of days of treatment varied with the test system.

The solid tumor systems were evaluated by measurement of tumor weight. The tumor weights are reported in grams for Walker 256 and in milligrams for S180 and Ca755. The ratio of the mean weight of the tumors in treated animals to that in controls ($T/C \times 100 = X$ percent) and the difference in mean body weight change of the animals in the treated and control groups is reported in table 1 for all groups with more than 65% survivors. For these tumors the second stage of sequential testing is done if the ratio of T/C of the first stage is at least equal to 0.44 for S180, Ca755, and Walker 256. For L1210 and P388, the mean survival time of the animals is calculated. The ratio of the mean survival time of the treated group to that of the control groups expressed in percent ($T/C \times 100$) and the difference in mean body weight change between Day 1 and Day 5 of the animals in the treated and control groups was determined for groups with more than 65% survivors on Day 5. With these tumors the second stage of sequential testing is done if the ratio T/C of the first stage is ≥ 1.25 .

The deaths were recorded for all groups. The maximum tolerated dose in an individual experiment is defined as the highest dose which produces not more than 2 deaths in 6 animals or not more than 3 deaths in 10 animals. With L1210, deaths before Day 6 are considered nonleukemic and form the basis for toxic evaluation. When a toxic result ($> 2/6$ or $3/10$ deaths) was observed, the test was repeated at an appropriately lower dose until the maximum tolerated dose was reached. If the T/C value in survival studies was less than 85% the dose was considered too high and was reduced in the next test.

The CCNSC quality control (4) was followed in the experiments. This includes the limitations of toxic deaths, the number of "no takes," and the mean tumor weight range (or survival time range) among control animals. It also includes the use of a positive control.

RESULTS AND DISCUSSION

The results of the screening of 7 species of medicinal plants used in the Philippines for the treatment of cancer are shown in table 1. The data are summarized according to the basic format of Abbott et al (1). In confirmation of previous screening of these species of plants collected in other areas, none of the plants collected in the Philippines met the CCNSC criteria of effectiveness for the L1210, P388, S180, Ca755, and Walker 256 screens; however, at the various dose levels tested extracts of several of the plants did show some indication of tumor inhibitory activity.

Extracts of *A. tagala* at a dose of 500 mg/kg injected ip and 500 and 1000 mg/kg injected sc yielded approximately a 10% increase in survival time in mice that had L1210. There was a slight decrease in the survival time of mice inoculated with P388 when treatment was given at 500 mg/kg, whereas at the same dose level there was 21% and 13% inhibition of tumor growth with S180 and Walker 256 respectively. As indicated by the weight change of the animals and the numbers of survivors of toxicity it is possible that higher doses of the extract might have been used.

Extracts of *C. zeylanicum* at dose levels up to 500 mg/kg were ineffective against L1210 and P388. At a dose of 250 mg/kg it inhibited S180 by 30% and Ca755 by 35%. The inhibitory effect against Walker 256 at the same dose was 19%. The extract appeared to be relatively nontoxic and higher doses might have been used.

Extracts of *C. melo* yielded only an 11% increase in survival time of mice with L1210 and were ineffective in the P388 and Ca755 systems. A dose level of 500 mg/kg resulted in a 22% reduction in tumor weight for S180 and a 30% reduction in tumor weight for Walker 256. The dose level of 500 mg/kg appeared to be somewhat toxic.

Extracts of *E. variegata orientalis* were ineffective in increasing the survival time of mice with L1210 or P388. S180 and Ca755 were inhibited 28% and 23% respectively; Walker 256 showed a 36% reduction in tumor size. Some weight loss in the animals was evident at dose levels of 500 and 1000 mg/kg indicating that maximum tolerated doses had been reached with this extract.

A dose level of 500 mg/kg of the extracts of *R. sativus* had no tumor inhibitory effect against L1210, P388, S180, or Walker 256. With the same dose level there was 28% inhibition of Ca755. A dose level of 1000 mg/kg of the extract increased the survival time of mice that had L1210 by 11%, with some suggestion of toxicity.

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Extracts of *V. odorata* yielded only a 10% increase in the survival time of mice L1210 and no increase in the lifespan of mice with P388. They were ineffective against S180 and essentially ineffective against walker 256 but did yield 32% inhibition for Ca755.

V. negundo yielded an 11% and 5% increase in the survival time of mice with L1210 and P388 respectively and was ineffective against Walker 256. There was 16% inhibition of S180 and 32% inhibition of Ca755.

S (PART 2)

VOL. 2, NO. 1, APRIL 1971

137

Table 1.- In vivo data on plant extracts

Botanical name	Family name	Host*	Test system†	Vehi- cle‡	Route of adminis- tration	Day of first in-jec- tion§	No. of in-jec- tions§	Day an- imals were killed	Dose (mg/kg. given once daily)	Surviv- vor	Wt dif- ference¶ (g)	Tumor wt or survival (T/C)**	Percent (T/C x 100)
<i>Aristolochia tagala</i>	Aristolochiaceae	2	L1210	D	ip	1	Z	died	125	6/6	-0.3	7/7 days	100
		2	L1210	D	ip	1	Z	died	250	6/6	-0.5	7/7 days	100
		2	L1210	D	ip	1	Z	died	500	6/6	-1.3	7.5/7 days	107
		2	L1210	D	sc	3	Z	died	500	6/6	0.4	10/9 days	111
		2	L1210	D	sc	3	Z	died	1000	6/6	-0.2	10/9 days	111
		1	S180	D	ip	1	7	8	500	5/6	-1.6	1046/1330 mg	79
		50	Walker 256	D	ip	3	4	7	500	6/6	-4.4	6.9/7.9 g	87
		2	P388	D	ip	1	10	died	500	6/6		10/11.5 days	87
		2	L1210	5	ip	1	Z	died	125	6/6	-0.9	8.0/8.5 days	94
		2	L1210	5	ip	1	Z	died	150	6/6	-0.8	7.5/8.0 days	94
<i>Cinnamomum zeylanicum</i>	Lauraceae	2	L1210	5	ip	1	Z	died	200	6/6	-0.9	8.0/8.0 days	100
		2	L1210	5	ip	1	Z	died	250	6/6	-3.1	8.5/8.5 days	100
		2	L1210	5	sc	3	Z	died	250	6/6	-2.7	9.0/9.0 days	100
		2	L1210	5	sc	3	Z	died	500	6/6	-0.1	9.0/9.0 days	100
		1	S180	5	ip	1	7	8	250	4/6		780/1110 mg	70
		2	Ca755	5	ip	1	11	12	250	9/9	0.2	1310/2030 mg	65
		3	Walker 256	5	ip	3	4	7	250	6/6	0.2	4.4/5.4 g	81
		2	P388	5	ip	1	10	died	250	6/6		11/12 days	95
		2	L1210	5	ip	1	Z	died	250	6/6	-0.8	8.5/8.5 days	100
		2	L1210	5	sc	3	Z	died	250	6/6	-0.7	10/9 days	111
<i>Cucumis melo</i>	Cucurbitaceae	2	L1210	5	sc	3	Z	died	500	6/6	-0.0	10/9 days	111
		1	S180	5	ip	1	7	8	500	6/6		870/1110 mg	78
		2	Ca755	5	ip	1	11	12	500	9/10	-0.9	2070/2030 mg	102
		3	Walker 256	5	ip	3	4	7	500	6/6	-5.2	3.8/5.4 g	70
		2	P388	5	ip	1	10	died	500	6/6		10/12 days	83
		2	L1210	5	ip	1	Z	died	500	6/6	-0.4	8/8 days	100
		2	L1210	5	sc	3	Z	died	500	6/6	-0.2	9/9 days	100
		2	L1210	5	sc	3	Z	died	1000	6/6	-0.4	9/9 days	100
		1	S180	5	ip	1	7	8	500	4/6		800/1110 mg	72
		2	Ca755	5	ip	1	11	12	500	10/10	-1.0	1570/2030 mg	77
<i>Erythrina variegata</i> Linn. var. <i>orient</i>	Leguminosae	3	Walker 256	5	ip	3	4	7	500	6/6	-3.3	3.4/5.4 g	64
		2	P388	5	ip	1	10	died	500	6/6		10/12 days	83

ORTS (PART 2)

VOL. 2, NO. 1, APRIL 1971

<i>Raphanus sativus</i>	Cruciferae	3	Walker 256	5	ip	3	4	7	500	6/6	-3.3	3.4/5.4 g	64
		2	P388	5	ip	1	10	died	500	6/6		10/12 days	83
		2	L1210	6	ip	1	Z	died	500	6/6	0.2	8.5/9 days	94
		2	L1210	6	sc	3	Z	died	500	6/6	-0.6	9/9 days	100
		2	L1210	6	sc	3	Z	died	1000	6/6	-1.0	10/9 days	111
		1	S180	6	ip	1	7	8	500	6/6	-2.3	1440/1330 mg	108
		2	Ca755	6	ip	1	11	12	500	10/10	0.2	1297/1805 mg	72
		50	Walker 256	6	ip	3	4	7	500	6/6	-7.0	8.9/7.4 g	121
		2	P388	6	ip	1	10	died	500	6/6		11/11 days	100
		2	L1210	6	ip	1	Z	died	250	6/6	-0.7	9/9 days	100
<i>Viola odorata</i>	Violaceae	2	L1210	6	sc	3	Z	died	500	6/6	-1.6	10/9 days	110
		2	L1210	6	sc	3	Z	died	1000	6/6	-1.5	10/9 days	110
		1	S180	6	ip	1	7	8	500	6/6	-3.4	1370/1330 mg	103
		2	Ca755	6	ip	1	11	12	500	10/10	-1.0	1224/1805 mg	68
		50	Walker 256	6	ip	3	4	7	500	6/6	-0.6	6.7/7.4 g	92
		2	P388	6	ip	1	10	died	500	6/6		10/11 days	91
		2	L1210	6	ip	1	Z	died	500	6/6	-0.1	9/9 days	100
		2	L1210	6	sc	3	Z	died	500	6/6	-1.2	10/9 days	111
		2	L1210	6	sc	3	Z	died	1000	6/6	-0.7	10/9 days	111
		1	S180	6	ip	1	7	8	500	6/6	-0.3	1120/1330 mg	84
<i>Vitex negundo</i>	Verbenaceae	2	Ca755	6	ip	1	11	12	500	10/10	0.4	1231/1805 mg	68
		50	Walker 256	6	ip	3	4	7	500	6/6	3.15	7.8/7.4 g	105
		2	P388	6	ip	1	10	died	500	6/6		11.5/11 days	105
		2	L1210	6	ip	1	Z	died	500	6/6	-0.1	9/9 days	100
		2	L1210	6	sc	3	Z	died	500	6/6	-1.2	10/9 days	111
		2	L1210	6	sc	3	Z	died	1000	6/6	-0.7	10/9 days	111
		1	S180	6	ip	1	7	8	500	6/6	-0.3	1120/1330 mg	84
		2	Ca755	6	ip	1	11	12	500	10/10	0.4	1231/1805 mg	68
		50	Walker 256	6	ip	3	4	7	500	6/6	3.15	7.8/7.4 g	105
		2	P388	6	ip	1	10	died	500	6/6		11.5/11 days	105

*1 = Swiss mice; 2 = BDF₁ mice; 3 = Sprague-Dawley rats; 50 = random bred albino rats.

†Ca755 = Adenocarcinoma 755; L1210 = lymphoid leukemia L1210; S180 = Sarcoma 180; Walker 256 = Walker carcinosarcoma 256 (im) P388 = Leukemia P388.

‡S = Alkali diluted with saline; 6 = corn oil; D = alcohol.

§Z = received injections until death.

||Number of animals surviving out of number started on tests as defined in individual protocols.

¶Average weight change of treated host minus average weight change of control host (exclusive of tumor weight).

**Tumor weight (mg or g): mean tumor weight of test animals (1)/mean tumor weight of control animals (C).

Survival (days): mean or median survival time of test animals/mean or median survival time of control animals.

REFERENCES

1. Abbott, B. J., Hartwell, J. L., Leiter, J., et al. Screening data from the Cancer Chemotherapy National Service Center Screening Laboratories. XLI. Plant extracts. *Cancer Res (supp)* 27 (No. 5, part 2):364-527, 1967.
2. Masilungan, V. A., Relova, R. N., and Raval, J. S. The anticancer activity of medicinal plants locally used in the treatment of cancer. *Philipp J Cancer* 93:57-65, 1965.
3. Quisumbing, E. Medicinal plants of the Philippines. Manila, Department of Agriculture and Natural Resources, Republic of Philippines, 1951, 1234 pp.
4. Cancer Chemotherapy National Service Center. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother Rep* 25:1-66, 1962.

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God Bless

Ann Wignmore and Viktoras Kulvinskis,

Prophets of Health and Spirit,

Whose Teachings

Have Given Birth

to a

New Generation of

Healers

SPROUT IT! THE BASIC TECHNIQUE

20

Of course, you can eat these sprouts before they mature, but you would be losing a lot. The popularity of sprouts is based on their reputation as nutritional superfoods. But this is not the case if they are not fully mature! Their nutritional peak usually occurs at the time of their first leaf division. Many restaurants serve a salad full of brown and yellow sprouts. The brown parts are the hulls which have not fallen off yet and the yellow represents the lack of full chlorophyll development. Simply speaking, you are not getting what you are supposed to. Not only that, certain undesirable factors remain present within the seed until the plant fully develops (see p. 113). Once you grow a delicious crop of mature green sprouts, you will never eat them any other way. Eating immature sprouts shortchanges you in total yield as well as nutrition. Patience pays.

What Seeds To Sprout

Your sprouter is ideal for growing indoor vegetable seeds that develop chlorophyll-rich, green leaves. These include:

Alfalfa	Garlic	China Red Pea
Clover	Onion	Turnip
Fennel	Mustard	Cabbage
Radish	Buckwheat	Broccoli
Kale	Sunflower	Chia

Which Seeds - Which Size - How Much

6" 2-3 Tbsp	8" 5 Tbsp	9" 6-7 Tbsp
Radish	Alfalfa	Buckwheat
Garlic	Clover	Sunflower
Onion	Fennel	China Red Pea
Cabbage		
Kale		
Turnip		
Chia		
Mustard		

SPROUT IT! THE BASIC TECHNIQUE

21

6 INCH BASKET, 2 - 3 TABLESPOONS SEED

These varieties are hot and/or spicy. Use the smallest 6 inch basket for them unless you have a spicy appetite. Use 2-3 tablespoons of seed: Garlic, Onion, Radish, Cabbage, Turnip, Kale, Broccoli, Mustard, Canola, Chia. Garlic and onion are delicious and very hearty. Mustard is hot. Cabbage, turnip, kale, broccoli and canola are all cabbage family. Chia is a gelatinous seed (see p. 157).

8 INCH BASKET, 5 TABLESPOONS SEED

Alfalfa, Clover and Fennel. Clover is a spicy cousin of alfalfa with bigger leaves. Fennel is a bitter herb and very healthy for the respiratory system. Use it mixed with alfalfa for best taste. 5 Tbsp can yield one pound of salad greens.

9 INCH BASKET, 6 - 7 TABLESPOONS SEED

Buckwheat, Black Skin Sunflower, China Red Pea. These three seeds represent the largest leaves and tallest stalks of the sprouting family. Mung beans may also be grown this way even though they are not a salad green. Choose only whole buckwheat and sunflower in the shell.

Double Decker Technique

Stack Your Sprouters Since space is often a problem, here's a technique to conserve it. Two sprouting baskets on top of each other take up less space than two side by side. During the first phase of germination (days 1-4), any two of the sprouters could be stacked with the smaller basket underneath the bigger one. Insert the double decker into the greenhouse.

It's a great space saver, but that's not all. Seeds send their roots vertically downward searching for soil. The extra height of the double decker gives the roots from the top basket plenty of room to stretch. Ordinarily, they are matted underneath the basket by the floor of the greenhouse tent. Elevating the basket gives the roots space to breathe and has the potential to increase the length of the stalks.

P. 03

NUTRITION

Fenugreek

Trigonella foenum-graecum

Fenugreek is actually a member of the legume (leguminosae) family. It is a cousin of clover and lucerne (alfalfa). The Pharaohs of Egypt used it in religious ceremonies. The monks of the Middle Ages used it to treat blood poisoning, failing eyesight, fevers, palpitations and kidney troubles. It is widely cultivated in Arab countries here it was traditionally used to stimulate appetite. Its chemical composition resembles that of cod-liver oil and is considered a 'herb' to garlic, enhancing that herb's disinfectant properties. It is a tonic because it is so rich in many minerals including sulfur and vitamin E. It 'feeds' the blood and is recommended for ailments that are associated with weakness such as anemia and infections. Both the seed and the whole plant are used.

Fenugreek is a demulcent meaning it is soothing to the mucous membranes and reduces inflammation. A tea made from the seed is used as a gargle and for sore throats. It also acts as an expectorant, clearing the mucosa of the chest and respiratory system. Fenugreek is used to increase their milk supply. Poultices made from the seed and leaves have been used on wounds, boils, sores and ulcers. The seed contains beneficial volatile oils and steroidal compounds which may be used to regulate blood cholesterol. Fenugreek sprouts have both the properties of the seed as well as the sprout. This sprout should be used to stimulate and to fortify.

1-41 Nutrition In Fenugreek Seed (in Milligrams per 100 grams) [30]

Iron	23.0	Zinc	2.50
Calcium	323.0	Niacin	1.64
Phosphorus	176.0	Iron	33.53
Protein	6.4	Arginine	2.47
Carbohydrate	191.0	Leucine	1.76
Fiber	296.0	Lysine	1.68
Water	770.0	Aspartic acid	2.71
	67.0	Glutamic acid	3.99

NUTRITION

Cabbage

Brassica oleracea

The cabbage family of foods includes Chinese cabbage, broccoli, kale, turnip, rutabaga, radish, mustard, rape, cauliflower, collard greens, brussels sprouts and kohlrabi. Of these, the first eight are good for home sprouting. Cabbage is rich in fiber and a good source of minerals especially potassium 253mg per 100 grams, sulfur 1710mg and vitamins C 47mg, E and A 200 IU. It has a drying and binding faculty that makes it effective for inflammations and hot swellings. Historically, cabbage was used to combat scurvy at sea even by the famous Captain Cook. Sailors would make sauerkraut from it which coaxed their intestinal tract with friendly bacteria and promoted regularity. The fermentation from the kraut remedied the complaints of flatulence that are common with the cabbage family. It is also improved by boiling and draining. European literature often mentions cabbage juice as the best medicine for hangovers. Philip Moore in the *Hopa of Health* in 1964 wrote, "the juice of cabbage purges the head, being put into the nostrils. Being taken after much drinking, it withstandeth drunkenness."

The cabbage family and other cruciferous vegetables are now taken seriously at the National Cancer Institute. Worldwide epidemiological studies consistently point to lower than average cancer rates for those groups regularly eating dark green leafy vegetables. The crucifers contain compounds called glucosinolates which block the development of cancer. Turnip greens contain between 59 and 166 milligrams per hundred grams of glucosinolates. When cooked, the concentration drops to a range of 21-94 (46).

Cabbage has the greatest potential in colon and stomach cancer. Several major epidemiological studies demonstrate that eaters of leafy green crucifers have the lowest rate of colon cancer. Other population surveys add cancers of the prostate, rectum, esophagus, lung and bladder to the list. In May 1978, Lee Wattenberg, M.D., a professor of pathology at the University of Minnesota Medical School, reported in the *Journal Cancer Research*, that he had isolated chemicals called indoles from cruciferous vegetables which were potent

121

NUTRITION

or we know that "free radicals" are the guilty party because chromosome breaks created in the presence of L-Carotene sulfate were prevented by the anti-oxidant superoxide dismutase [16].

Raw and sprouted vegetables contain enzymes that oppose tumor growth. Tumors release enzymes called proteases which break down healthy tissue around the tumor and increase potential tumor growth. Inhibiting enzymes in live foods called protease inhibitors, block the actions of these proteases and the spread of the tumors. Sprouted seeds and beans, particularly soybeans and lima beans, are our finest dietary sources of these protective enzymes [23].

Flaxseeds and their young sprouts are one of our best dietary sources of the essential omega-3 fatty acids such as alpha-linolenic acid. Freshly sprouted 1-2 day flaxseeds provide an excellent source of this extremely unstable oil. Studies show that the omega fatty acids have an inhibiting effect on tumor growth [24]. Specifically, they decrease the synthesis of prostaglandins thus decreasing the migratory ability of tumor cells and metastasis [25].

Sprouts also show promise to help in the fight against breast cancer. Soybean sprouts are nature's finest source of plant isoflavones which are converted in our stomachs to isoflavone equol. High estrogen levels stimulate breast tumor growth, but research shows isoflavone equol to have excellent anti-estrogenic qualities similar to that of cruciferous vegetables [26].

In 1992, researchers at Johns Hopkins University Medical School isolated sulphoraphane, a compound found in broccoli and other brassica family vegetables. Sulphoraphane stimulates a cell's production of certain protective enzymes that resist tumor growth [9]. Studies of cancer patterns in the U.S. and abroad reveal strong statistical linkage between the consumption of raw vegetables and relative immunity to a variety of cancers. Researchers have long known that cells exposed to carcinogens respond by generating an assortment of highly effective enzymes that guard against malignant growth. They

NUTRITION

Common black pepper contains nearly 10% (by weight) piperine. Piperine is related to azofole which causes cancer in mice. Should therefore deduce that black pepper, a condiment on nearly every dining table in America, causes cancer in humans? Aflatoxin is one of the most potent carcinogens known and just hearing its name is alarming to the public. It can be a contaminant in moldy bread, cheese, corn, peanuts and fruit, but it is extremely rare. Asparagines and nitroso compounds are suspected causes of stomach and digestive tract cancers. Beets, celery, lettuce, spinach, radish (rhubarb) all contain 200 milligrams or more of nitrates (per 100 portion). Should we incriminate these common vegetables, consumed for thousands of years across multi-national and cultural borders because chemical components isolated within them have demonstrated mutagenic effects on rats?

Anti-Oxidants & Anti-Carcinogens

All right. Nature is not benign. Natural toxins do exist. But natural foods and particularly sprouts, also contain numerous beneficial enzymes, anti-oxidants and anti-carcinogens such as vitamin E, beta-carotene, selenium, super-oxide dismutase and ascorbic acid (vitamin C) that act as the body's defense mechanism against toxins whether natural or man-made.

Beta-Carotene is found in mature alfalfa sprouts and in all plants contain chlorophyll. It is a very efficient free radical trap [17]. It has demonstrated anti-carcinogenic activity in rats and mice [18]. Vitamin E significantly inhibits skin, liver, colon, and mammary tumors in experimental animals by a variety of carcinogens [19]. Athletes, rich in foods containing the sulfur amino acids, are rich in anti-oxidants and anti-mutagens and may even be effective against potent aflatoxins [20]. Vitamin C (ascorbic acid) was shown to nullify carcinogenic in rodents treated with ultraviolet radiation and nitrosamines. Mushrooms like shiitake contain the active polysaccharide compound lentinan. Lentinan stimulates interferon production. Lentinan is a powerful anti-tumor agent [27].

NUTRITION

appear to work by bonding with the toxins and preventing their chemicals from reaching the cell's vulnerable genetic material. Then, they flush them from the body. The most effective enzyme stimulated by the sulphoraphane in cabbage family foods is called quinine reductase. Sulphoraphane, by the way, is related to mustard oil. Foods that contain sulphoraphane are cabbage, broccoli, kale, cauliflower, turnip, Chinese cabbage, collard greens, brussels sprouts, and even non-cruciferous vegetables like carrots, green onions, chives and the sprouts of broccoli, kale, turnip, garlic, onion and Chinese cabbage.

Chlorophyll, one of the most basic nutritional elements in plants, is a well known blood purifier and, in fact, is similar in chemical structure to human hemoglobin. Numerous animal studies demonstrate that chlorophyll can be converted into hemoglobin. Alfalfa sprouts are one of our best dietary sources of earth grown chlorophyll. (Algae from lakes is highest.)

Alfalfa sprouts have also demonstrated a remarkable cholesterol reducing capacity. Studies in both humans and a wide selection of animals including dogs, rabbits, chickens, pigeons and pigs have shown a regression of atherosclerosis (40) and a considerable drop in the levels of serum cholesterol. Saponins in alfalfa appear to be responsible for lowering cholesterol and balancing the bile (41). They create a soothing action that prevents cholesterol and bile salts from being absorbed. Although there has been concern in the past about the toxicity of saponins, research showed positive results in the lack of toxicity of alfalfa saponins in monkeys and rats (42).

Enzymes are protein-like chemical agents that facilitate all life-building processes such as digestion, absorption and metabolism. The enzyme and anti-oxidant super-oxide dismutase, abundant in sprouts especially green sprouts like alfalfa, obstructs the free radical-oxanavine-alfalfa pathology. In a 1980 report published in *Human Genetics*, chromosome breaks caused by free radicals were prevented by the anti-oxidant super-oxide dismutase (16). In a 1993 study at the Indiana University School of Medicine, 78 female mice

NUTRITION

received a lethal dose of 580 rads of x-radiation designed to cause extreme free-radical activity. Half of the 23 placebo-fed mice died within 30 days. The remaining 55 mice were fed supplements made from wheat sprouts. All of them survived except one. Wheat sprouts are high in the pre-cursor enzyme that stimulates the body's manufacture of super-oxide dismutase (35).

Wheat sprouts have also demonstrated anti-mutagenic activity in mice and rats in three separate studies. Members of the flavonoid family, shatofide and swertisine, both glycosides of apigenin appear responsible for the wheat sprouts' strong anti-mutagenic behavior. (38) The sprouts were not grown to the grass or green stage.

Perhaps because of their rapid germination and protein manufacture, sprouts are also rich sources of nucleic acids. Nucleic acids are the genetic keys to protein and tissue growth found in the cytoplasm, nucleus and chromosomes of cells. They resist cell mutation and promote healthy cell growth. These results indicate that sprouts have a profound effect on our ability to fend off free-radical induced diseases such as cancer and immune system disorders.

Now for the Real Carcinogens

Rather than isolating and attacking natural toxins in plants which are balanced by a multitude of enzymes and nutrients, perhaps we should turn our efforts to eliminating known carcinogens in our environment. Free oxygen radicals are caused by numerous dietary and lifestyle factors including medical drugs, air and water pollution, pesticides, alcohol, cigarettes, fried foods, smoked and barbecue foods, nitrates, even good old toast and coffee.

Charred meats and rancid fats should not be part of anyone's diet. The heating of proteins and fats creates a variety of DNA damaging agents (22). So does the caramelization of sugars and amino acids visible on the browned ends and crust of common toast bread. In fact, the amount of burnt and browned material in the human diet may be several grams per day. In comparison, a 2 part

B1

SPROUTS

To Grow and Eat

Esther Munroe

THE STEPHEN GREENE PRESS
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1-F44

EXHIBIT A

4 / Sprouts—A Food Bargain

Wheat and rice sprouts have a sweet nut-like flavor; alfalfa, rye and clover sprouts have a fresh-green taste not unlike that of other salad greens; radish and mustard sprouts are somewhat peppery and should be used in conjunction with more bland foods; sprouts from seeds of the cabbage family—broccoli, Brussels sprouts, cauliflower and cabbage itself—taste rather like the parent plants; sprouts from each one of the many kinds of peas, beans and lentils have their own distinctive tastes and are particularly versatile in cooking.

For greatest food value, all of the edible sprouts may be eaten raw. All may also be cooked in many different ways, which are covered in the recipe section of this book. If sprouts are new to you and your family, it is suggested that you try several different kinds, both raw and cooked. You are certain to find some, and perhaps many, that you enjoy.

Anyone who likes vegetables—either raw or cooked—will find a whole new world of taste treats in sprouts. If you are careful not to mention the “this is good for you” aspect, most children will also like many sprouts.

Warning—only chemically untreated and certified edible seeds should be used for sprouting.

Getting Your Seeds

As mentioned earlier and as most home gardeners are already well aware, by far and away the largest percentage of seeds sold for planting have been treated with some chemical or other—the list is long and constantly changing. These chemicals are chiefly pesticides used to protect the seeds from various infestations. However, in some cases, for instance alfalfa and red clover, methyl dyes are used to indicate foreign origin. The latter, and many of the former, are highly toxic to human beings and, to make matters worse, accurate warnings are not always required to appear on the packaging. As a result, for safety's sake, *sprouts from seeds that have been treated with any chemical whatsoever have to be considered not fit for human consumption.*

One way to get seeds for sprouting is through the mail. A number of mail-order seed companies do sell a selection of untreated seeds. At the end of this book there is a partial list of such suppliers. However, to be on the safe side, always specify when ordering that you want only untreated seeds—seeds completely free of chemicals and certified edible. And be sure to double check when your seeds arrive. This is more than worth the little effort involved.

Since more people have become interested in natural and/or untreated foods, many health food stores have begun to stock untreated seeds that are suitable for sprouting. If there is no natural food store available in your area, you may wish to consult the list of suppliers at the back of the book for the names of health food supply houses from whom you can order by mail. Once again, be sure to specify that you want untreated, edible seeds suitable for sprouting.

Most stores that specialize in Oriental foods also sell seeds for sprouting—particularly mung bean and soybeans, both of which are widely used in Far Eastern dishes.

6 / Getting Your Seeds

In addition, many chain stores and supermarkets, as well as the corner market, sell brown rice, whole peas, beans and lentils of various kinds that are perfectly safe for sprouting. These seeds can sometimes be used successfully, although they are often not as satisfactory as those intended primarily for sprouting. They may well contain among them cracked or broken or too-old seeds that will not sprout. However, it is worth a try, especially if you can find a small store that doesn't treat its stock too roughly.

Anyone who lives in a rural area may be able to get some untreated seeds from the local grain dealer. Here again it is essential to be sure that the seeds are suitable for human consumption.

For the home gardener there is yet another alternative. Even though you cannot eat treated seeds, it is possible to plant those seeds to grow a crop of your own seeds that are safe to eat. Just select a few plants that seem particularly suitable and allow them to go to seed. Use no chemicals on the plants. Pick the seeds when they are fully mature, dry them completely and store in closed containers in a cool, dry, dark place. You then have your own untreated, fully wholesome seeds for sprouting—at almost no cost.

Warning—only chemically untreated and certified edible seeds should be used for sprouting.

Which Sprouts for What

Before going into the particulars about each sprout, there are a few generalities to bear in mind. Sprouts are always tastiest when young and fresh (in fact, they should rarely be allowed to reach over 1 inch in length). So it is best to sprout only a limited number of each variety at a time and to try to plan to have one crop eaten before the next harvest is ready.

Following is an alphabetical listing of the most commonly sprouted seeds, offering in a nutshell the specifics of recommended sprout length and sprouting time, plus general suggestions for use, for each sprout. Also see the "quick reference" table placed for convenience just before the recipe section. This table gives seed quantities and their expected sprout yield, plus handy information on growing and cooking (if any) times.

Once again, it is important to remember that the sprouting times given here are average times and may vary with the age of the seed, its moisture content and with the humidity and room temperature (some people feel that even the content of the water used affects the sprouting process). As a result, do not be bound by the exact times listed but rather by the length of the sprouts, being sure to harvest them before they pass their peak.

ADZUKI BEAN. These tiny red-brown beans are not as well known in the Western world as they deserve to be. In the Orient they have been grown for centuries and are often used in dishes for festive occasions. Easy to sprout, they are ready to eat in 4 or 5 days, at a sprouted length of ½ to 1 inch. Use adzuki bean sprouts in any recipe that calls for mung bean, soybean or any other legume sprouts.

8 / Which Sprouts for What

ALFALFA. The name for this forage crop is Arabic, meaning "a fine, green fodder" and it derives from the fact that the Arabs discovered their horses grew stronger and more fleet on this crop than on any other. Sprouted for only 1 or 2 days, to a sprout length of 1/8 inch, alfalfa sprouts are particularly good in pastries, cereals and appetizers. If the sprouts are grown 4 or 5 days to about 1 inch and exposed to sunlight for a few hours, which allows them to develop chlorophyll, they make a delicious addition to fresh green salads. Alfalfa is one of the easiest of all seeds to sprout and, while the seed is fairly expensive, the yield is high, so the resulting crop of sprouts is quite reasonable in price.

ALMOND. Unhulled almonds are not easy to find but, if you do locate some in a health or Oriental food store, they are delicious sprouted and used as you would any nut meat. Soak for twice as long as other seeds—about 24 hours, rinse often and keep quite wet. A sprouting time of 3 to 5 days will give you 1/8 to 1/4 inch sprouts, which are just right for use.

BARLEY. This is one of the oldest of all known grains, its origin is lost in man's own prehistoric beginnings. Barley formed a part of the religious rites for many Old World peoples. Once a mainstay in bread making, its use today is largely confined to the brewing of alcoholic beverages and to livestock feed. However, barley sprouts have a fine nut-like flavor that makes them suitable for use anywhere you would use wheat, oats or rice—particularly in breads, soups and casserole dishes. Treat as you would wheat, oats or millet sprouts. Sprouting time is 3 to 5 days; use when sprouted length is no longer than the seed.

BEANS—Black, Broad, Fava, Kidney, Lima, Navy, Pea, Pinto and Red (see also **MUNG BEANS** and **SOYBEANS**). The bean kingdom is one of the most varied in the plant world and beans range in size from limas and kidneys, which are nearly an inch long, to pea beans, no more than 3/8 inch long. Almost every country has some traditional dish made with beans and, by the same token, all have their body of folklore about beans, even to the extent of thinking of them as unlucky. Under most conditions the bean is a prolific producer and the peoples of

Which Sprouts for What / 9

South America and the Orient still rely on beans as a staple item of diet. Sprouted beans lose the gas-producing quality of the unsprouted bean and become readily digestible. Each variety of bean sprout has a distinctive taste and all are most adaptable to every kind of use—in appetizers, breads, drinks, main dishes, salads and soup. Most of the bean sprouts listed here are as good raw as they are cooked. Sprouting time for most beans is 3 to 5 days and sprouted length should be 1/2 to 1 1/2 inches, depending on the bean. A good rule of thumb for beans is "the larger the bean, the shorter the sprout." Larger bean sprouts tend to be tougher and smaller ones more tender, so try different lengths for each bean and select the length and flavor you prefer.

BROCCOLI see **CABBAGE FAMILY**

BRUSSELS SPROUTS see **CABBAGE FAMILY**

BUCKWHEAT. Buckwheat is one of the fastest growing of all grain or cereal crops. For centuries it was used throughout Russia, Manchuria and Europe in bread making. It is less extensively grown in the United States than in the past, which is unfortunate because it is almost totally free of disease or blight. Most of the American crop is used in pancake flour and livestock feed, while buckwheat honey is relished for its distinctive taste and dark color. Buckwheat kernels tend to stick together, so rinse rather than soak them and sprinkle often to keep moist. Sprouting time is rather short—2 to 4 days usually. Some people prefer their buckwheat sprouts no longer than the grain itself—1/4 to 1/2 inch—and others like a sprout 3/4 to 1 inch long. Buckwheat sprouts can be used in any recipe that calls for barley, millet, oat, rice or wheat sprouts, e.g., breads, cereals, main dishes and soups.

CABBAGE FAMILY—Broccoli, Brussels sprouts, Cabbage, Cauliflower, Collards and Kale. The cabbage family has almost as many relatives as the bean family. All are easy to sprout and each one produces a tasty sprout of a slightly different flavor. Not everyone likes every kind of sprout from the cabbage family, so experiment with a few seeds at a time. Sprouting time is 3 to 5 days for a sprouted length of 1/2 to 1 inch. One word of caution, these sprouts tend to become strong flavored or bitter if grown too long, so use them when they are most pleasant to

14 / Which Sprouts for What

your taste in soups, salads and main dishes. Like their parent plants, they are high in vitamins and so are well worth sprouting.

CAULIFLOWER see CABBAGE FAMILY

CHIA. Chia seeds come from one of the family of sage plants and are not well known outside of Mexico and the American Southwest. Nor are they easy to sprout, being somewhat gluey so that they stick together when moist. However, aficionados rave about the slightly pungent taste they add to salads and sandwich spreads and point to their high trace-mineral content. So, if you are able to locate chia seeds and decide to try them, do so in moderation. But don't try to soak them; they will stick together in an unmanageable mass. Put the seeds on a saucer or small plate, sprinkle with water and let stand overnight. Another way is to put a small amount of water on a plate and float the seeds on top. Sprinkle again as the seeds dry out. Sprouting time is usually 1 or 2 days for 1/8- to 1/4-inch sprouts, which is the best length for most uses.

CHICKPEA. The chickpea, as it is called in the United States, is known by many names elsewhere in the world, garbanzo being one of the most common. The plant is highly drought resistant, which makes it ideal for growing in the subtropics, as well as the drier sections of Europe and North America. Nearly, although not quite, as high in protein as soybean sprouts, chickpea sprouts can be used in any dish that calls for the former. Chickpeas should also be sprouted the same way as soybeans—rinsing about 4 to 6 times in 24 hours because they tend to spoil quickly if left wet for any length of time without rinsing. Sprouting time is generally about 5 to 8 days and sprouted length about 3/4 to 1 inch.

CLOVER. The red clover seed is the one you want for sprouting. Handle it the same way as alfalfa and use it in the same kind of recipes; i.e., when the sprouts are just the length of the seed, they are best for appetizers, cereals and breads but when grown to 1-inch length and greened in sunlight use them in salads.

CORN. Untreated corn seed is almost never available, so two possibilities are open to the sprouter. Buy the whole field corn used for animal

Which Sprouts for What / 15

feed or raise your own sweet corn and let some of it mature for drying and later sprouting. The latter course will give you the best product. For sprouting, many people prefer the variety of corn known as Deaf Smith County but any sweet corn that you enjoy fresh will be palatable as sprouted corn. Try adding corn sprouts to soups or casseroles. Steam some and serve buttered as a side-dish vegetable. Oven-dried and finely ground corn sprouts may be used to replace part or all of the cornmeal in a quick-bread recipe. The possibilities are limited only by the inventiveness of the cook. Sprouting time can vary from 3 to 8 days, depending on the variety of seed used. Sprouted length should be 3/4 to 1 inch.

CRESS. A fast-growing plant with a peppery taste, its leaves are most often used in sandwiches or salads. Cress sprouts may be used in the same way but with moderation because of their pungency. Somewhat gluey like chia seeds, cress seeds should be sprouted the same way and harvested when the sprout is about 3/4 to 1 inch long, usually after 2 to 4 days.

FENUGREEK. This member of the legume family is almost unknown in the Western hemisphere but in the Far East it is used for seasoning, particularly in curry powder. The seeds sprout readily and in 3 or 4 days will reach 1/2-inch length, which is just about right to bring out Fenugreek's spicy flavor. Any longer in the sprouting process and the sprouts get bitter tasting.

FLAX. Flax is one of man's most helpful folk remedies for use in poultices and cough syrups, while the fiber is used to make linen. Flax seed is slightly gluey and should be sprouted like chia. Grown to 3/4- or 1-inch length—a length which usually takes 3 or 4 days of growing time—flax sprouts make a mild-flavored and delicate addition to salads and soups. If desired, they may be grown somewhat longer and greened in the sunlight for 3 or 4 hours to be used as you would any salad greens.

GARBANZO see CHICKPEA

LENTIL. One of the oldest vegetables known to man, lentils are mentioned in the Bible as the food for which Esau sold his birthright to Jacob. There are many different strains of lentil, ranging in color from green to yellow and redish brown. They sprout easily and even those